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TITLE: Determination of a Unique Pattern of Gene Expression in Node Positive Breast Cancer Using Serial Analysis of Gene Expression (SAGE)

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13. ABSTRACT (Maximum 200 Words)

The main hypothesis of this project is that a specific genetic expression pattern for lymphogeneous spread exists in a small fraction of cells in primary breast cancer. The Principal Investigator (PI) believes that this expression pattern will be the same in primary tumors from different women. These patterns could then be exploited to determine the lymph node status of a primary breast cancer. This DOD concept award extension investigated whether a genetic signature in the primary tumor could be developed for lymph node positive breast cancer. Using a combination of serial analysis of gene expression (SAGE) and microarray techniques, a preliminary genetic signature has been developed. Further experiments are in progress to refine the genetic signature and confirm the overexpression of certain genes that predict for lymph node positive breast cancer.

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Progress report: Determination of a Unique Pattern of Gene Expression in Node Positive Breast Cancer Using Serial Analysis of Gene Expression

Principal Investigator: Adam Brufsky, MD, PhD

Introduction:

Genome wide molecular expression profiling has demonstrated the capacity to separate heterogeneous malignancy diagnosis into new more precise diagnostic subcategories. Gene expression profiling may have the capacity to differentiate primary breast cancer with or without metastatic potential. Such differentiation would allow enhance tailoring of current treatments and improved design of clinical trials. Estrogen receptor expression has been found to be associated with a pattern of gene expression involving increased or decreased expression of hundreds, possibly thousands of transcripts. It is thus critical in any exploration of genetic expression of Invasive Ductal Carcinoma to study estrogen receptor expression phenotype subtypes individually.

Body:

Our study is analyzing 2-5 cm primary breast tumors for the presence of a metastatic phenotype. Estrogen receptor, progesterone receptor and HER-2/neu negative samples were selected for the initial phase of this study. Nine samples were chosen for highest quality extracted RNA from 28 tissue bank tumor specimens. These nine include five with either positive sentinel or axillary lymph nodes and four with negative lymph node status.

Affymetrix gene chip analysis using U133A chips have been performed in duplicate on these initial nine specimens. Candidate dysregulated genes have been identified by relative expression difference and cluster analysis (Abstract; ASCO, 2003 # 3483). Non parametric statistical analysis using permutation testing suggests a measurable phenotype distinction between lymph node associated primary tumors and those not associated with lymph node spread.

The Serial Analysis of Gene Expression(SAGE) assay, an open ended mRNA expression transcript assay has been completed. Over 400 identified transcript tags have associated predictive scores of 75% or greater in differentiating tumors associated with lymph node spread from those without. (Abstract: San Antonio Breast Cancer Symp [SABCS], 2003 #563).

Microarray scanning has been initiated on an additional set of forty similar tumors (2-5cm,IDC, [ER/PR/HER2neu neg]). This set of gene expression libraries will allow for testing a predictive profile generated from thirty tumors onto a test set of ten tumors. In addition, reverse transcriptase PCR (RT-PCR) is being performed to validate the ten most robust dysregulated gene products identified by SAGE and Affymetrix analysis described above. Primers have been obtained to first validate the transcripts in the initial nine samples and if validated to prospectively test their class predictor potential in the additional forty specimens. Specimens of the next closest phenotype(ER/PR neg, Her2/neu pos) are being collected to proceed with similar

testing to this next subset should a measurable genetic signature of lymph node spread be confirmed in the initial subset described above.

Key Research Accomplishments:

- Developed genetic profile of lymph node positive breast cancer using SAGE
- Refined genetic profile of lymph node positive breast cancer using cDNA microarrays
- Began RT-PCR profiling of genes predictive of lymph node positive breast cancer

Reportable Outcomes:

Hergenroeder P, Peters D, Handley D, Dabbs D, O'Hare E, Suppe B, **Brufsky A.** Towards a genetic signature of lymph node positive breast cancer. Proc ASCO 2003; 22:3483a.

Hergenroeder PF, Peters DG, Handley D, Lyons-Weiler J, Dabbs D, **Brufsky AM.** Building a gene expression predictive classifier of lymph node positive breast cancer. Br Can Treat Reports 2003; 22:563a

Conclusions:

In the extension year of this concept award, we have met the research objectives of the award. We have developed a genetic profile predictive of lymph node positive breast cancer both by serial analysis of gene expression (SAGE) and c DNA microarray analysis. Further experiments will refine this predictive classifier, and confirm the genes which comprise the classifier. An NIH R01 grant is currently in preparation to perform a blinded analysis on 200-300 consecutive primary breast cancer specimens to attempt to predict lymph node status.